Acute Onset Human Atrial Fibrillation Is Associated With Local Cardiac Platelet Activation and Endothelial Dysfunction

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Objectives

The purpose of this study was to determine whether acute onset atrial fibrillation (AF), independent of other risk factors, predisposes to an early prothrombotic state.

Background

Several risk factors predispose to the hypercoagulable state in human AF, but whether acute onset AF alone is prothrombotic remains unclear.

Methods

Patients with paroxysmal AF (n = 22) underwent radiofrequency catheter ablation. All patients presented in sinus rhythm. Baseline blood samples were obtained simultaneously from the femoral vein (systemic sample) and the coronary sinus (local cardiac sample). The AF was induced by burst atrial pacing in 14 patients (AF group). A control group (n = 8) underwent atrial pacing at 120 beats/min. Blood samples were recollected after 15 min. Platelet P-selectin expression (CD62) was measured using flow cytometry. Markers of thrombin generation (thrombin antithrombin complex, prothrombin fragment 1.2), inflammation (C-reactive protein, interleukin-6), and nitric oxide were measured using enzyme-linked immunosorbent assays.

Results

Neither local nor systemic platelet activation changed in the control group. In the AF group, local cardiac platelet activation (percent P-selectin [↑] platelets) increased significantly (2.2 ± 0.6% to 2.8 ± 1.0%, p = 0.007); however, systemic platelet activation did not change. The AF group had increased local thrombin generation (thrombin antithrombin complex: 8.5 ± 7.6 ng/ml to 33.2 ± 17.4 ng/ml, p = 0.003; prothrombin fragment 1.2: 95.6 ± 45.6 μmol/l to 243.8 ± 120.1 μmol/l, p = 0.003), decreased nitric oxide production (25.2 ± 10.8 μmol/l to 22.3 ± 10.0 μmol/l, p < 0.02), and no change in inflammatory markers.

Conclusions

Human AF causes local cardiac platelet activation within minutes of onset. The results demonstrate how AF alone, independent of other risk factors, may contribute to the hypercoagulable state. (J Am Coll Cardiol 2008; 51:1790–3) © 2008 by the American College of Cardiology Foundation

Whether atrial fibrillation (AF) itself causes platelet activation remains unresolved. Although several risk factors (e.g., age, hypertension, diabetes mellitus, heart failure) predispose to stroke in patients with AF, it has been controversial whether the arrhythmia itself independently leads to platelet activation and hypercoagulability (1–6). Studies have yielded conflicting results because heterogeneity in AF burden and patient profile make it difficult to dissociate the effect of AF on prothrombotic markers from the effect of other risk factors. Furthermore, it is unclear to what degree paroxysmal AF leads to hypercoagulability. The prothrombotic effects of acute AF likely develop on a cardiac level before manifesting in the peripheral circulation, so examination of serological markers in peripheral blood may not reflect the local cardiac events.

We hypothesized that acute onset human AF leads to platelet activation on a local cardiac level, independent of other risk factors.

Methods

The study was approved by the Institutional Review Board of Loyola University Medical Center. Written informed consent was obtained from all subjects.

Study protocol. All patients (both groups) had a history of paroxysmal AF but were in sinus rhythm at the beginning of the study. The patients presented for radiofrequency AF ablation. Exclusions included left ventricular dysfunction, rheumatic valve disease, mitral valve prolapse, or any valvular regurgitation more than mild/physiologic. Sheaths were inserted in the femoral veins and coronary sinus (via right internal jugular vein). A quadripolar catheter was positioned in the high right atrium for pacing.
During baseline sinus rhythm, 10 ml of blood was simultaneously obtained from the peripheral femoral venous sheath (systemic sample) and the coronary sinus sheath (local cardiac sample). Atrial fibrillation was induced by burst atrial pacing (cycle length 180 ms) in 14 consecutive patients (AF group). Following 15 min of AF, simultaneous local and systemic samples were drawn again. No heparin or other medication was administered during this time.

The fast ventricular response observed during AF in these patients (mean 121 beats/min) could theoretically affect the hemostatic system independent of the arrhythmia itself. To assess the effect of the ventricular rate, a control group (n = 8) underwent atrial pacing at 120 beats/min for 15 min instead of burst atrial pacing. These patients also had a history of paroxysmal AF. This produced a ventricular rate equivalent to the AF group; however, the control patients were not in AF. Local and systemic blood samples were drawn before and after pacing. All samples were immediately transferred for analysis in citrated tubes. The investigators were blinded to platelet function results.

Assessment of platelet activation. Platelet P-selectin expression (CD62) was studied using 2-color whole blood flow cytometry. Citrated whole blood samples were fixed in 1% paraformaldehyde for 30 min and then pelleted, resuspended in Tyrode’s buffer, and labeled with CD61-FITC and CD62-PE (BD Biosciences, San Jose, California). The degree of platelet activation was determined as the fraction of CD62(+) events that was also CD62(+).

Serological markers of thrombosis, inflammation and endothelial function. Serological markers were measured using commercially available enzyme-linked immunosorbent assays. Samples were run in duplicate and read at 450 nm.

Thrombosis. Thrombin antithrombin complex (TAT) and prothrombin fragment 1.2 (F1.2) were measured (Dade-Behring, Deerfield, Illinois). Both TAT and F1.2 are independent in vivo markers: TAT of thrombin generation and F1.2 of antithrombin consumption. The TAT complexes form covalently following thrombin generation, and F1.2 is formed by conversion of prothrombin to thrombin.

Inflammation. Inflammation is associated with thrombin generation, platelet activation, and the pathophysiology of AF. High-sensitivity C-reactive protein and interleukin-6 were measured (American Diagnostica, Greenwich, Connecticut, and BD Biosciences, San Jose, California, respectively).

Endothelial function. Quantification of nitric oxide (NO) production was performed using a standard assay (R&D Systems, Minneapolis, Minnesota), which relies on conversion of nitrate to nitrite and detection as an azo dye product of the Greiss reaction (7).

Statistics. Data are shown as mean ± standard deviation. Comparisons between groups were performed by 2-sample t test, and within each group by paired sample t test. Statistical significance was defined as p < 0.05.

Results

Patient characteristics. Patient characteristics are shown in Table 1. The following medications were withheld ahead of the procedure: clopidogrel and aspirin—7 days, warfarin—5 days, enoxaparin—24 h, antiarrhythmic medications—3 days. More patients in the control group were female and had a history of clopidogrel use; however, clopidogrel had been discontinued >30 days and thus did not affect platelet function. Baseline blood pressure was similar in both groups and did not change after 15 min of either AF or pacing.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline Characteristics of the Patients</th>
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<tbody>
<tr>
<td></td>
<td>Atrial Fibrillation (n = 14)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>54 ± 12</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>93</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>7</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>36</td>
</tr>
<tr>
<td>Stroke/transient ischemic attack (%)</td>
<td>0</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>56 ± 6</td>
</tr>
<tr>
<td>Left atrial size (mm)</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>113 ± 20/68 ± 16</td>
</tr>
<tr>
<td>After 15 min</td>
<td>114 ± 17/77 ± 18</td>
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<tr>
<td>Medications (%)</td>
<td></td>
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<tr>
<td>Warfarin</td>
<td>36</td>
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<tr>
<td>Clopidogrel</td>
<td>0</td>
</tr>
<tr>
<td>Aspirin</td>
<td>36</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>29</td>
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<tr>
<td>Calcium-channel blocker</td>
<td>7</td>
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<td>Digoxin</td>
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Local and peripheral platelet activation in acute AF. Both peripheral and local cardiac platelet activation were similar at baseline in the AF and control groups. As shown in Figure 1, local cardiac platelet activation increased significantly after 15 min of AF; however, it did not significantly change with pacing. In contrast, Figure 2 demonstrates that peripheral platelet activation remained stable and did not change following either AF or pacing.

Local thrombin generation. We hypothesized that the platelet activation was due to increased thrombin generation. Local TAT and F1.2 levels were measured at baseline in sinus rhythm, after 15 min of AF (no heparin), and then at the conclusion of the AF ablation after the administration of intravenous heparin (to provide an internal control by inhibiting thrombin).

As shown in Figures 3A and 3B, local TAT and F1.2 levels increased significantly after 15 min of AF, indicating ongoing thrombin generation due to the arrhythmia. As expected, intravenous heparin reduced thrombin generation to baseline levels.

Inflammation and endothelial dysfunction. We hypothesized that local cardiac thrombin generation and platelet activation in acute AF were secondary to an inflammatory response. However, cardiac C-reactive protein (1.10 ± 0.77 μg/ml vs. 1.14 ± 0.78 μg/ml, p = 0.15) and interleukin-6 (3.7 ± 8.3 pg/ml vs. 3.5 ± 8.0 pg/ml, p = 0.4) did not change following 15 min of AF.

Reduced NO production due to endothelial dysfunction is a known cause of platelet activation and thrombosis. Cardiac levels of total NO decreased following 15 min of AF (25.2 ± 10.8 vs. 22.3 ± 10.0, p < 0.02), indicating that acute onset AF is associated with endothelial dysfunction. There was no change in cardiac NO production in the control group.

Discussion

Platelet activation and AF. This study demonstrates that acute onset AF is associated with activation of platelets and other prothrombotic markers. These events occur on a local cardiac level before manifesting in the peripheral circulation and are due to AF per se, not to the fast ventricular rate seen during the arrhythmia (8).

One study (9) suggested platelet activation was time-dependent in AF; however, other studies (1–6) yielded conflicting results. This is partly due to use of peripheral blood samples from patients with different AF durations. Platelet activation, an early thrombotic event, is expected to occur on a cardiac level before manifesting in the peripheral circulation in acute AF. Hence, contrary to permanent AF, peripheral serological markers may not reflect intracardiac events (10). Furthermore, the exact AF burden in previous studies was heterogeneous, thereby potentially confounding results. The present study is the first to examine the effect of a precise AF duration on local cardiac prothrombotic events and to demonstrate that AF per se causes platelet activation.

Endothelial damage/dysfunction, inflammation, and AF. Although inflammation may contribute to AF pathogenesis and hypercoagulability, the present data indicate that platelet activation in acute AF may not be related to inflammation.

Experimental AF is associated with abnormal blood flow, shear stress, and NO bioavailability leading to increased expression of prothrombotic markers (4,11). The present study demonstrates reduced cardiac NO associated with acute human AF, implying that local endothelial dysfunction may be a cause of platelet activation. Because platelet activation is an early prothrombotic event, this effect starts on a local cardiac level before manifesting peripherally. This hypothesis needs further clinical and experimental verification.

Study limitations. The 15-min AF duration was dictated by the desire not to prolong the ablation unnecessarily.
Future studies need to delineate the exact time course of hemostatic system activation. This study examined platelet activation change in individual patients following a defined intervention. Though the absolute degree of platelet activation after 15 min of AF was similar to the control group baseline, it is the magnitude of change that is important. The trend toward decreased cardiac platelet activation with pacing does not have a physiological explanation and is likely coincidental.

Coronary sinus blood reflects the total cardiac, not only the left atrial, pool. Although local cytokines were not secreted after 15 min of AF, an inflammatory process is not excluded.

This study indirectly assessed NO production using the Greiss reaction. Demonstration of human cardiac endothelial dysfunction in acute AF is difficult and requires experimental verification with direct NO measurements.

Conclusions

Acute onset AF causes platelet activation on a local cardiac level. This may provide a mechanism explaining how short episodes of AF predispose to stroke especially in patients with underlying vascular disease and supports the practice of anticoagulating patients with AF and stroke risk factors, even if the AF is paroxysmal.

Acknowledgments

The authors thank Jean Delpriore, RN, Cynthia Finn, RN, and Jillian Bianchi, BS, for their efforts.

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REFERENCES